quantitative descriptions of DNA elasticity. Here we connect well-defined force-extension measurements with a novel structure-guided model for DNA elasticity, the twistable worm like chain model. This analytical description incorporates the essential physical characteristics of DNA, including how its helicity depends on extension. In addition, at forces exceeding ~65 pN, when DNA overstretches and melts, our experimental assay exposes rich features that can be fully attributed to the underlying base sequence. An equilibrium thermodynamic model is presented that quantitatively captures this melting behaviour solely based on the knowledge of DNA sequence and elasticity. These results offer a new standard description for the mechanics of DNA and enable deeper quantitative insight into the physical interactions of DNA-associated proteins.

400-Pos Board B200
Underwound DNA Under Tension: Structure, Elasticity and Sequence-Dependent Behavior
Maxim Sheinin, Scott Forth, John F. Marko, Michelle D. Wang.
Torque-induced separation of DNA strands plays an important role in a wide variety of cellular processes, such as transcription and replication. In the present work, we investigate mechanical properties of torsionally-melted DNA at the single-molecule level using an angular optical trap. While applying a constant tension to the DNA molecule, we simultaneously measure the extension change and torque as the DNA is being underwound up to and beyond the end of the melting phase transition. We find torsionally-melted DNA to be left-handed and flexible to bending, but with a relatively high torsional resilience. It is shown that our data can be incorporated into the recent DNA phase transition model. We have also discovered that at biologically relevant low forces, sequence has a significant impact on the underwound DNA properties. Implications of our findings for the global DNA force-torque phase diagram are discussed.

401-Pos Board B201
Sequence Dependent Structural Transitions in DNA Induced by Torque
Florian C. Oberstrass, Louis Fernandes, Zev Bryant.
B-DNA becomes unstable under superhelical stress and is able to adopt a wide range of alternative conformations including Z-DNA, cruciforms, and strand-separated DNA. Localized sequence-dependent destabilization of superhelical DNA is thought to be important for the regulation of biological processes requiring strand separation, such as transcription and replication initiation. To directly probe the effect of sequence on structural transitions driven by torque, we have measured the torsional response of a panel of DNA sequences using newly developed extensions of the rotor bead tracking assay[1]. In these assays, a submicron rotor bead is employed as a rotational probe attached to the side of a single stretched DNA molecule. Plots of torque as a function of twist show clear signatures of sequence-dependent cooperative structural transitions. Our results shed new light on the structure and stability of mismatched DNA sequences as well as the torsional properties of replication origins.
1. Bryant, Z. et al. *Nature* Vol. 424 338-41 (2003).

402-Pos Board B202
Insights Into Sequence Dependent Effects on DNA Elasticity Using Single Molecule Techniques
Krishnan Raghunathan, Yih-Fan Chen, Joshua Milstein, Benjamin Juliar, Justin Blaty, Jens-Christian Meiners.
The mechanical properties of DNA play an important role in regulating gene expression within a cell. Sequence does not only code, but also affects structural properties of the DNA. These variations can change the intrinsic curvature and the stiffness of the DNA. To study the effect of sequence on elasticity, it must be decoupled from curvature effects. For this aim, we designed two DNA constructs with similar curvature but different sequences and measured their elasticity in single-molecule stretching experiments with optical tweezers. We report substantial differences in their persistence length. We complement these experiments with studies on the effect of these differences in elasticity on protein-mediated DNA looping as a means of transcriptional control, using the Lac repressor as a model system.

403-Pos Board B203
Stretching Poly(A) to Investigate Elastic Behavior at Low Ionic Strength
Bennett S. Kalafut, Koen Visscher.
In addition to serving as the genetic template for protein synthesis in the ribosome, RNA folds in sequence-dependent ways to form structures that have regulatory or catalytic functions. Homopolymeric polyadenylic acid (poly(A))is known to form, due to base-stacking, single-stranded helices in solutions with ionic strength high enough to screen the self-repulsion of RNA's negatively charged backbone (e.g. 500 mM Na+), giving it elastic properties different than those of a random RNA sequence. Understanding the behavior of these helical sequences is necessary, for example, to in turn understand the folding of A-rich regions of riboswitches or the binding of poly(A) binding protein to mRNA's poly(A) tail.
When stretched using optical tweezers, well-screened poly(A) shows a clear helix-coil transition, manifested as a shoulder or plateau on the force-extension curve. By stretching poly(A) to sufficiently high force in low ionic strength solutions, we can probe the relative strengths of backbone repulsion and base stacking and moreover investigate the role of possible tight or diffuse binding of di- and polyvalent cations, thus furthering our understanding of helix formation in poly(A) sequences.
We have found that the helix-coil transition is less distinct at 100 mM Na+ than at 500 mM Na+ and totally absent at 10 mM Na+. These results and their implications, and additionally the effect of the divalent cation Mg++ on elasticity of poly(A), will be presented.

404-Pos Board B204
Probing DNA Stiffness with Magnetic Tweezers
Qing Shao, Sachin Goyal, Laura Finzi, **David D. Dunlap**.
The exceptional stiffness of DNA is routinely attributed to base stacking and repulsion between neighboring, negatively charged phosphates. Furthermore, well established biochemistry and recent single molecule experiments show that small, charged molecules and intercalating agents can dramatically alter the pitch and twist of the double helix. It is very likely that cells and macromolecular complexes have evolved to influence these phenomena in order to alter the flexibility of DNA to efficiently catalyze DNA transactions. In order to gain insight into how cells manipulate DNA efficiently, a quantitative understanding of DNA elasticity is necessary. Using magnetic tweezers to twist and stretch single DNA molecules, experiments were performed to probe the parameters of DNA stiffness. Although this technique is exquisitely sensitive and permits broad exploration of twist versus extension data, accurate models with which to interpret the stiffness parameters are critical. In particular, the knee point of the DNA extension versus twist curve, which is sensitive to both the bending and torsional rigidity of the molecule, is a signature, which if modeled accurately might give insight into how base stacking and electrostatics contribute to DNA stiffness. Our experiments showed that diaminopurine substitution for adenine, which adds an additional hydrogen bond to AT base pairs, stiffens DNA by about 50% without significantly changing the knee point. Instead adding low molecular weight polycations such as spermine or spermidine to the solution appeared to soften DNA and promote plectoneme formation at lower values of torsion. Thus base pair stability and, implicitly, stacking seem to have affected only the DNA elasticity while charge neutralization also favored the conversion of excess twist into writhe.

405-Pos Board B205
Synthesis and Single-Molecule Characterization of a DNA Hairpin Construct Based on the TAR RNA Sequence
Catherine A. Dietrich, Xiaowen Wang, Micah J. McCauley, Mark C. Williams, Megan E. Núñez.
Optical tweezers are used to examine the folding and unfolding of single molecules, revealing the effects of force, ionic strength, proteins, and small molecules on the kinetic and thermodynamic parameters of bimolecular stability. Here, we examine a DNA hairpin construct related to the trans-activation responsive region (TAR) RNA sequence of HIV-1. In an optical tweezers experiment, a bead at one end of a duplex DNA assembly is attached to a micropipette, while a bead at the other end is held in an optical trap, thus allowing investigators to relate picoNewton forces necessary to unfold the DNA with corresponding nanometer changes in length. Force extension/relaxation data are used to elucidate DNA folding and unfolding processes.
Our DNA construct design contains a central hairpin structure with long double-stranded "handles" attached to both the 3' and 5' end of the hairpin. The DNA ''handles'', each approximately 3 kb in length, were synthesized by PCR using biotin- and digoxigenin-labeled primers that serve to attach the final construct to 5 μm-diameter beads for use with the optical tweezers. The smaller central hairpin sequence was obtained via solid-phase organic synthesis. After purification and assembly with a linker oligonucleotide, the 5' handle and the hairpin structure were ligated together and purified by agarose gel electrophoresis. This product was then ligated to the 3' handle and again purified. Individual molecules of the final hairpin construct, approximately 6 kb in length, were bound to streptavidin or anti-digoxigenin-labeled beads and their unfolding behavior was studied using optical tweezers.

406-Pos Board B206
Single Molecule FRET Microscopy of Immobilized DNA Molecules: Keeping Track of Dye Integrity and Stoichiometry
Victoria Birkedal, Asger C. Krüger, Flemming Besenbacher.
Fluorescence Resonance Energy Transfer (FRET) at the single molecule level is a powerful technique for studying conformational changes of biomolecules. Dual color excitation schemes help sorting the single molecule data and quantifying FRET efficiencies within a single molecule. Here, we compare several data analysis methods for accurate FRET measurements and for discriminating